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# THE EFFECTS OF ORAL ADMINISTRATION OF DIFFERENT DOSES OF HYDROALCOHOLIC EXTRACT OF SILYMARIN ON STATUS OF SERUM TRACE ELEMENTS

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## ABSTRACT

In this research, the effect of oral administration of different doses of hydroalcoholic extract of silymarin on the status of trace elements with antioxidant properties (Se, Cu, Zn, Mn and Fe) was studied. Fifty male rats were randomly used in five groups including healthy control group (treated by saline), diabetic control group (treated by saline) and three diabetic groups that were daily treated by different oral dose of silymarin (100, 175 and 250 mg $kg^{-1}$). Diabetes was induced by intraperitoneal injection of streptozotocin (50 mg $kg^{-1}$) in rats. Rats were orally treated for 14 days by gavage. Weight was measured before STZ injection, 7 and 14 days after treatment. The blood glucose was measured before injection of STZ, 3 days after injection and 7 and 14 days after diabetes was induced (under treatment by silymarin). At the end of the study, blood samples were taken by heart puncture. Results showed that the average weight increased during the 14 days of treatment with silymarin and the blood glucose was decreased. Hydroalcoholic extract of silymarin increased trace elements levels in treated groups by dose-dependent manner. The present study showed that in diabetic rats, high therapeutic doses of silymarin had a major effect on serum antioxidant factors. Results also showed that hydroalcoholic extract of silymarin increased the body weight and decreased serum glucose concentration at the end of the treatment period. Silymarin in a dose dependent manner could improve trace element levels in diabetic rats. Therefore, it may cause reduction of oxidative stress in the diabetic rats.

**Keywords:** Silymarin, Diabetes Mellitus, Trace Elements, Antioxidant, Rat

## 1. INTODUCTION

Silymarin is a flavonoid found in the herb milk thistle Silybum marianum. A standardized extract obtained from the seeds of S. marianum was found to contain approximately 70-80% of the silymarin flavonolignans and 20-30% chemically undefined fraction, comprised mostly of polymeric and oxidized polyphenolic compounds (Ghosh *et al*., 2010). Silymarin is a standardized extract of flavonolignans silybinin, isosilybinin, silydianin and silychristin and other minority compounds from the seeds of Silybum marianum (Valenzuela and Garrido, 1994).

The main feature of diabetes mellitus is chronic hyperglycemia, which leads to the disturbance of carbohydrate, fat and protein metabolism. Even though diabetes is classified as a single disease, various secondary complications may occur such as renal failure,

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cardiac abnormalities, diabetic retinopathy, neuropathy, atherosclerosis and neuropathic pain (Nazıroglu *et al*., 2012). Owing to the increasing prevalence of diabetes, multidisciplinary study aimed at preventing and treating diabetes is a world-wide research priority (Kaneko *et al*., 2008). Several micronutrients have beneficial effects in healthy subjects and also in diabetes. Selenium, copper, zinc, manganese and iron are essential components of metalloenzymes such as Se-cys containing glutathione peroxidase, Cu/Fe cytochrome C oxidase or different types of superoxide dismutases. All of these trace elements are important in intra- and extra-cellular antioxidant defense (Flores *et al*., 2011). The role of trace elements in the various metabolic processes and their importance in healthy and diseased humans has attracted many researchers to study the trace elements status in normal and pathological conditions.

Type 1 diabetes mellitus is a disorder of glucose metabolism associated with reduction in insulin production and utilization by tissues (Kaneko *et al*., 2008). The disturbance in trace elements status and increased oxidative stress in diabetes mellitus may contribute to the insulin resistance and development of diabetic complications (Basaki *et al*., 2012). Selenium, being an integral part of glutathione peroxidase, has a protective role against tissue damage caused by peroxides produced from lipid metabolism. Selenium deficiency in humans causes decreased glutathione peroxidase activity and cardiomyopathy. In Se-deficient rats, insulin secretory reserve was significantly reduced and glucose intolerance developed in rats maintained on selenium and vitamin E-deficient diets (Mooradian and Morley, 1987). Ruiz *et al*. (1998) reported that the mean plasma selenium and copper levels in diabetic patients were significantly lower than the control group. Under conditions of Cu deficiency, several components of the oxidant defense system can be compromised. Predictably, the activities of CuZn-SOD and ceruloplasmin are sensitive to tissue Cu as these enzymes require Cu as a catalytic cofactor. Moreover, Cu deficiency can alter other Reactive Oxygen Species (ROS) scavengers including metallothionein (a Cu and Zn containing protein) and the non-protein thiol, glutathione (Uriu-Adams and Keen, 2005). Basaki *et al*. (2012) reported that copper levels in diabetic patients were lower than the control group. Kubisch *et al*. (1994) showed that the activity of SOD and tolerance of pancreatic beta cells to oxidative stress due to diabetic risk factors was increased. Uriu-Adams and Keen (2005) reported that the concentration of copper in diabetic patients compared to healthy subjects was increased.

Zn plays a role in insulin synthesis, storage and secretion by pancreatic islet cells. Zn may stimulate energy consumption in skeletal muscle and brown adipose tissue and may increase the pancreatic insulin content and improve the glucose tolerance test (Faure *et al*., 1992). Zinc is also effective as an antioxidant (Basaki *et al*., 2012). Isbir *et al*. (1994) demonstrated a 20% decrease in serum Zn in type I diabetes mellitus, apparently due to hyperzincuria. Mn is an important component of Superoxide Dismutase (SOD), an antioxidant enzyme which scavenges oxygen free radicals (Saber and Syed, 1999). Kosenko (1965) reported serum Mn level in diabetic cases is approximately one-half that of normal ones. In contrast, Lisun-Lobanova (1963) found elevated Mn levels in diabetic patients (Mooradian and Morley, 1987). Iron plays a main role in the decomposition of hydrogen peroxide in catalase (Madiwale and Liebelt, 2006). Fe is a highly prooxidant molecule and is able to generate Reactive Oxygen Species (ROS). It has been demonstrated that Fe concentrations in patients with diabetic retinopathy are higher than in normal subjects (Basaki *et al*., 2012). Effect of silymarin on kidneys of rats suffering from alloxan-induced diabetes mellitus was investigated (Soto *et al*., 2010). Haddad *et al*. (2011) reported that silibinin have antioxidant and hepatoprotective effects in rats with nonalcoholic steatohepatitis.

Interrelation between the inhibition of glycolytic flux by silibinin and the lowering of mitochondrial ROS production in perifused rat hepatocytes was documented (Detaille *et al*., 2008). According to abovementioned researches, the aim of study was to evaluate the effects of oral administration of different doses of hydroalcoholic extract of silymarin on the status of trace elements with antioxidant properties (Se, Cu, Zn, Mn and Fe).

## 2. MATERIALS AND METHODS

### 2.1. Animal Ethics

This experiment was accomplished under the approval of the state committee on animal ethics, Shiraz University, Shiraz, Iran. Also, we used the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes.

## 2.2. Silymarin Extraction

Silymarin seed was provided from Pakanbazr of Isfahan. Each 100 g of seed was sieved 3 times and the powder was mixed with 400 mL of ethanol 90%. After 48 h the liquid was passed through a number 40 sieve papers, the scum was then mixed with ethanol 70% and again sieved; after 24 h these two solutions were mixed. After distillation stage by evaporator, using a freeze dryer the extraction was dried and changed to powder. 3-4 grams of pure extraction of silymarin was obtained from 100 g of silymarin powder.

## 2.3. Study Groups

About 50 male Sprague Dawley rats from the animal lab of Shiraz University of Medical Sciences were provided in this study. They were divided to 5 groups, two were control groups (healthy and sick) and the others were orally (gavage) treated by pure silymarin in different doses (100, 175 and 250 mg $kg^{-1}$ of rat body weight). Diabetes was induced in 4 groups of rats (diabetes group and 3 treated groups) using IP injection of Streptozotocin (STZ) at a dosage of 50 mg $kg^{-1}$ of body weight. At the beginning of the study, body weight and glucose concentration were measured and at the end (14 days after treatment) blood sampling and serum separation were done. The sera were stored at -20°C until analysis.

## 2.4. Analysis Method

For measuring trace elements, 0.5 mL of serum and 0.5 mL of digesting solution (nitric acid + percholeric acid (70:30)) were mixed and sera were digested at 90°C in water bath for 16-20 h. Then the volume of the samples was increased to obtain primarily volume (1mL), by distilled water. Cu, Zn and Fe were measured in AIR-$C_2H_2$ (Flame) by atomic absorption spectrophotometry (Shimadzu AA-670, Kyoto, Japan). Se and Mn were measured by GFA-4B (Graphite Furnace Atomizer, Shimadzu) condition (Schmidt *et al*., 1986; Takahashi *et al*., 2000).

## 2.5. Statistical Analysis

The statistical analysis was performed using ANOVA followed by the Duncan test. Results are expressed as the mean±S.E.M. P-Values of 0.05 or less were considered statistically significant.

# 3. RESULTS

In the present study, doses of 100, 175 and 250 mg $kg^{-1}$ of hydroalcoholic extract of silymarin were used. On the 7th and 14th days of the experiment, body weight decreased in the diabetic groups ($p<0.05$) (**Table 1**). Body weight of the diabetic control group showed a significant decrease ($p<0.05$) (**Table 1**). Body weight in the fifth group, between one and fourteen days, a significant increase was observed ($p<0.05$) (**Table 1**). Blood glucose levels in diabetic control group in the third day after STZ injection into fourteen days after treatment had increased significantly ($p<0.05$) (**Table 2**). Blood glucose levels in fourth and fifth groups, between third day after the STZ injection and fourteen day after treatment showed a significant decrease ($p<0.05$) (T**able 2**). In the groups treated with silymarin the blood glucose level was decreased after 14 days.

The selenium values showed a significant increase in the group 5 compared to the other groups ($p<0.05$).

Evaluation of copper values showed that the group treated with dose of 250 mg $kg^{-1}$ silymarin had a significant increase in comparison to healthy control group ($p<0.05$). The mean value of copper in the treatment group with dose of 250 mg $kg^{-1}$ silymarin was increased compared to other groups ($p<0.05$).

Evaluation of zinc values showed that diabetic control group had a significant decrease compared to healthy control group ($p<0.05$), but showed no significant differences with healthy control group (**Table 3**).

**Table 1.** Effect of treatment with silybum marianum seed hydroalcoholic extract for 14 days on weigh

| | $Weight_1$ (gr) | $Weight_7$ (gr) | $Weight_{14}$ (gr) |
|---|---|---|---|
| Healthy control | $198.3±2.15^{a,A}$ | $223.2±2.65^{a,B}$ | $264.9±2.64^{a,C}$ |
| Diabetic control | $206.8±3.49^{a,A}$ | $191.3±2.7^{b,B}$ | $171.5±2.23^{b,C}$ |
| Diabetic+sily 100 mg $kg^{-1}$ | $207.8±6.49^{a,A}$ | $216.9±7.16^{a,A}$ | $222.7±8.51^{c,A}$ |
| Diabetic+sily 175 mg $kg^{-1}$ | $200.6±2.55^{a,A}$ | $220.8±3.41^{a,B}$ | $240.1±7.74^{d,C}$ |
| Diabetic+sily 250 mg $kg^{-1}$ | $206±6.09^{a,A}$ | $219.5±6.40^{a,AB}$ | $229.7±3.9^{cd,B}$ |

Dissimilar letters in each column indicate significant differences is between different groups ($p<0.05$)
Different capital letters in each row indicate significant differences between different groups ($p<0.05$)
One day before STZ injection ($Weight_1$), seven days after induction of diabetes ($Weight_7$) and fourteen days after induction of diabetes ($Weight_{14}$)

**Table 2.** Effect of treatment with silybum marianum seed hydroalcoholic extract for 14 days on glucose levels

| | Glucose1 (mg/dL) | Glucose3 (mg/dL) | Glucose7 (mg/dL) | Glucose14 (mg/dL) |
|---|---|---|---|---|
| Healthy control | $138.9\pm3.46^{a,A}$ | $139.8\pm5.7^{a,A}$ | $128.5\pm2.95^{a,A}$ | $125\pm3.02a,^{A}$ |
| Diabetic control | $138.1\pm2.81^{a,A}$ | $427.5\pm11.79^{b,B}$ | $447.8\pm13.5^{b,B}$ | $504.7\pm18.36^{b,C}$ |
| Diabetic+sily 100 mg $kg^{-1}$ | $139.3\pm2.54^{a,A}$ | $437\pm19.06^{b,B}$ | $434.2\pm33.76^{b,B}$ | $433\pm34.71^{c,B}$ |
| Diabetic+sily 175 mg $kg^{-1}$ | $136\pm4.31^{a,A}$ | $464.9\pm25.42^{bc,B}$ | $427.1\pm20.35^{b,BC}$ | $374.3\pm18.35^{c,C}$ |
| Diabetic+sily 250 mg $kg^{-1}$ | $134.7\pm4.03^{a,A}$ | $502.5\pm17.05^{c,B}$ | $478.1\pm32.1^{b,BC}$ | $422.9\pm20.61^{c,C}$ |

Dissimilar letters in each column indicate significant differences between different groups ($p<0.05$)

Different capital letters in each row indicates significant differences between different groups ($p<0.05$)

One day before STZ injection ($Glucose_1$), three days after injection of STZ ($Glucose_3$), seven days after induction of diabetes ($Glucose_7$) and fourteen days after induction of diabetes ($Glucose_{14}$)

**Table 3.** Comparison of serum levels of selenium, copper, zinc, manganese and iron in different groups of rats at the end of the test

| | Se (ng/mL) | Cu (µg/mL) | Zn (µg/mL) | Mn (ng/mL) | Fe (µg/mL) |
|---|---|---|---|---|---|
| Healthy control | $341.215\pm33.46^{a}$ | $0.863\pm0.04^{abc}$ | $1.334\pm0.26^{a}$ | $14.053\pm1.03^{a}$ | $10.57\pm1.27^{a}$ |
| Diabetic control | $334.695\pm25.66^{a}$ | $0.591\pm0.05^{b}$ | $0.667\pm0.12^{b}$ | $14.459\pm0.79^{a}$ | $7.347\pm1.03^{b}$ |
| Diabetic+sily 100 mg $kg^{-1}$ | $324.848\pm27.65^{a}$ | $0.917\pm0.09^{abc}$ | $1.241\pm0.14^{a}$ | $16.743\pm1.08^{ab}$ | $8.49\pm0.83^{ab}$ |
| Diabetic+sily 175 mg $kg^{-1}$ | $338.879\pm28.81^{a}$ | $1.014\pm0.14^{c}$ | $1.269\pm0.14^{a}$ | $16.867\pm0.74^{ab}$ | $6.673\pm0.81^{b}$ |
| Diabetic+sily 250 mg $kg^{-1}$ | $477.685\pm10.66^{b}$ | $1.476\pm0.03^{d}$ | $1.342\pm0.09^{a}$ | $18.429\pm0.88^{b}$ | $7.374\pm0.34^{b}$ |

Dissimilar letters in each column indicate significant differences is between different groups ($p<0.05$)

Evaluation of manganese value showed that healthy control group had significant differences with group under treatment with dose of 250 mg $kg^{-1}$ silymarin ($p<0.05$). The group under treatment with dose of 250 mg $kg^{-1}$ silymarin showed a significant increase compared to the diabetic control group ($p<0.05$).

Evaluation of iron value showed significant decrease in diabetic witness group compared to healthy control group ($p<0.05$).

Evaluation of zinc and iron in the diabetic witness group has shown a significant decrease in comparison with the healthy control group ($p<0.05$).

## 4. DISCUSSION

The present study shows injection of streptozotocin in rats causes diabetes mellitus and a decrease in the body weight. This point is in agreement with the findings of other reports (Greene and Lattimer, 1983; Kim *et al*., 2006; Mazloomi *et al*., 2010; Musabayane *et al*., 1995; Ramesh and Pugalendi, 2006; Sajedianfard *et al*., 2013). In the diabetic control group, from the first day until the end of the study weight loss was 24.5%. At the end of the treatment, in the silymarin groups an overweight percentage of 7 to 17% was observed. In this study, STZ injection in rats leads to an increase in the blood glucose. A 15% increase was observed in the blood glucose of diabetic control group. In groups with different doses of silymarin a decrease in blood glucose from 8 to 20% was observed. Dose of 250 mg $kg^{-1}$ silymarin could not reduce blood glucose more than 20% in the five groups.

Medicinal plants act with different mechanisms such as increased insulin sensitivity, enhanced glucose-dependent insulin secretion, stimulating regeneration of pancreatic islets, reduction of inflammation and oxidative stress in diabetic rats (Sezik *et al*., 2005). Silymarin by inhibited interleukin 1β and interferon γ, prevents the production on nitric oxide. Therefore, the prevent of the beta-cell dysfunction on humans (Matsuda *et al*., 2004). The mechanism of action of silymarin may have an effect on the kinetics of glucose-6 phosphatase and inhibition of gluconeogenesis, increase insulin and improve pancreatic function, in addition to reducing hydroxy-3-methyl-glutaryl-CoA reductase and improving liver function. Silymarin acts by inhibiting hepatic glucose-6-phosphatase hydrolysis in perfused rat hepatocytes by an inhibitory effect on glucose- 6-phosphatase and gluconeogenesis (Guigas *et al*., 2007). Silymarin recovers pancreatic function to increase insulin level. It seems silymarin is able to increase insulin level and improve pancreatic function and structure (Soto *et al*., 2004). Since trace elements are cofactors of antioxidant enzymes, their deficiency may be associated with increased oxidative stress through reduction in antioxidants and increased lipid peroxidation (Basaki *et al*., 2012). It is known that selenium plays a significant role in glutathione peroxidase, lipooxigenase pathway, organic antioxidant systems and vitamin A, E and C (Navarro and Lopez, 2000). Silymarin regulates the cytochrome P450 3A2 and glutathione peroxides in the liver of streptozotocin-induced diabetic rats (Malekinejad *et al*., 2012). In the present study, a

4.5% decrease in serum selenium levels was observed in the diabetic control group. Ruiz *et al*. (1998) reported that the mean plasma selenium levels were significantly lower in diabetic patients. Selenium levels in type-1 diabetic children were higher than normal children (Gebre *et al*., 1984). In the present study, only in the group treated with 250 mg $kg^{-1}$ silymarin was a 30% increase in the selenium concentration observed. Copper is involved in the protection of oxidative stress in diabetic people (Uriu-Adams and Keen, 2005). Between healthy control and diabetic control groups no statistically significant differences were observed. In this study, the concentration of copper in the 175 mg $kg^{-1}$ silymarin group compared to diabetic control group showed 41.5% increase and in the group treated with 250 mg $kg^{-1}$ silymarin a 60% increase was observed. In this respect, Mooradian and Morley (1987) reported that disturbance in glucose tolerance can be associated with secondary copper deficiency.

The concentration of zinc in the diabetic control group showed 50% reduction compared to the healthy control group. The concentration of zinc in the group treated with 250 mg $kg^{-1}$ silymarin showed an increase of 50.5%. Levine *et al*. (1983) reported zinc deficiency in diabetic rats. Faure *et al*. (1992) reported that zinc was decreased in pancreatic tissue of type 1 diabetic rats, but this appears to be normal in type 2 diabetic rats. Reduction of serum zinc levels in diabetic patients is due to high urine loss of zinc (Chausmer, 1998).

Manganese is a constituent of many enzymes involved in fat and protein metabolism and is utilized by various antioxidant enzymes such as Mn Superoxide Dismutase (MnSOD) and glutamine synthetase (Taylor *et al*., 2006). In this study, the concentration of manganese in the diabetic control group showed a 3% increase compared to the healthy control group. The concentration of manganese in the group treated with 250 mg $kg^{-1}$ silymarin showed an increase of 21.5%. Blood manganese levels in diabetic patients have been reported to be high, low, or unchanged (Walter *et al*., 1991). Manganese reduction was reported in diabetic patients (Kosenko, 1965; Walter *et al*., 1991; Kazi *et al*., 2008). In contrast, Lisun-Lobanova (1963) reported increasing levels of serum manganese in diabetic patients.

Iron has a major role in decomposition of hydrogen peroxide (Madiwale and Liebelt, 2006). The mean level of iron in the diabetic rats showed 30.6% reduction, but this level did not change in the silymarin groups. One reason for the reduction of iron in diabetic rats could be due to elevated levels of manganese. Manganese could have interfered with iron metabolism and inhibited iron absorption; therefore, the concentration of iron in tissues and blood was decreased. High requirements of the trace elements and their greater excretion lead to a decrease in the concentration of the trace elements in diabetic rats (Basaki *et al*., 2012). Interference of some of the trace elements such as manganese with iron could have increased the iron concentration in diabetic rats. The alteration of the mineral status in the diabetic rats may be due to the well-recognized cytokine response to vascular damage (Walter *et al*., 1991).

## 5. CONCLUSION

The present study showed that, high therapeutic doses of silymarin had a major effect on serum antioxidant factors in diabetic rats. It also showed that hydroalcoholic extract of silymarin increased the body weight and decreased serum glucose concentration at the end of the treatment period. Silymarin in a dose dependent manner could improve trace element levels in diabetic rats and therefore reduce oxidative stress in the diabetic rats.

### 5.1. Conflict of Interest Statement

We declare that we have no conflict of interest.

## 6. ACKNOWLEDGMENT

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